
CpG Oligodeoxynucleotides

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Summary

CpG oligodeoxynucleotides (ODNs) are a novel pharmacotherapeutic class with profound immunomodulatory properties. The mechanisms through which these agents work are incompletely understood, but it is clear that the oligonucleotides bind with both cytoplasmic and nuclear factors. In murine models, CpG ODNs can both prevent and treat the Th2-mediated inflammatory responses associated with asthma and atopy. This class of compounds is poised for human trials in asthma and allergy.

CpG ODNs: Discovery and Definition

CpG ODNs are a recently described class of pharmacotherapeutic agents that are characterized by the presence of an unmethylated CG dinucleotide in specific base-sequence contexts (CpG motif). These CpG motifs are not seen in eukaryotic DNA, in which CG dinucleotides are suppressed and, when present, usually methylated, but are present in bacterial DNA to which they confer immunostimulatory properties [1]. These immunostimulatory properties include induction of a Th1-type response with prominent release of IFN- γ , IL-12, and IL-18. CpG ODNs (18–24 bp in length) possess immunomodulatory properties similar to bacterial DNA.

Mechanism of Action of CpG DNA

Although there are cell surface proteins that can bind DNA, the immune stimulatory effects of CpG DNA require cell uptake [1, 2]. This DNA uptake is inducible and varies with cell type, but is sequence independent [3, 4]. Uptake probably involves both pinocytosis and endocytosis [4]. Although the nature of the intracellular receptor for CpG DNA is not yet clear, its intracellular location of the CpG receptor seems logical if this detection pathway evolved as a defense against intracellular pathogens. Several intracellular signaling pathways appear to be acti-

vated by CpG DNA. Within 7–10 min, the mitogen-activated protein kinases p38 and the c-Jun NH₂-terminal kinase are activated in human and murine B cells and macrophages [5, 6]. In addition, there is rapid generation of reactive oxygen species, which appears to be important in the activation of NF- κ B [7, 8].

These signaling pathways converge on the nucleus, resulting in the activation of multiple transcription factors and increased expression of multiple inflammatory messengers [9–15]. Overall, the most highly produced cytokines in the response to CpG are the Th1-like cytokines such as IL-12, IL-18 and IFN- γ . However, CpG DNA also induces B cells to produce IL-10, which appears to act in a counter-regulatory fashion to limit the inflammatory response to CpG [14].

Need for Immunomodulatory Therapy

In recent years, the paradigm of asthma therapy has shifted from symptomatic relief of bronchospasm to treatment of airway inflammation. Despite this increased attention to the role played by inflammation in the pathogenesis of asthma, the prevalence, severity and morbidity ascribed to asthma have grown over the past two decades [16]. Immunotherapy, or the use of allergen and/or immunomodulating agents to alter the immune response to an antigen, is a potentially curative therapy. Development of better immunotherapy protocols has been hindered by the relatively low potency of therapeutic allergens and the risk of serious and potentially fatal adverse reactions. If more effective and safer immunotherapy were available for the treatment of asthma, this would be a significant tool in the armamentarium of the clinician: patients could develop clinical tolerance to exposure to allergens, reducing their need for chronic anti-inflammatory therapy.

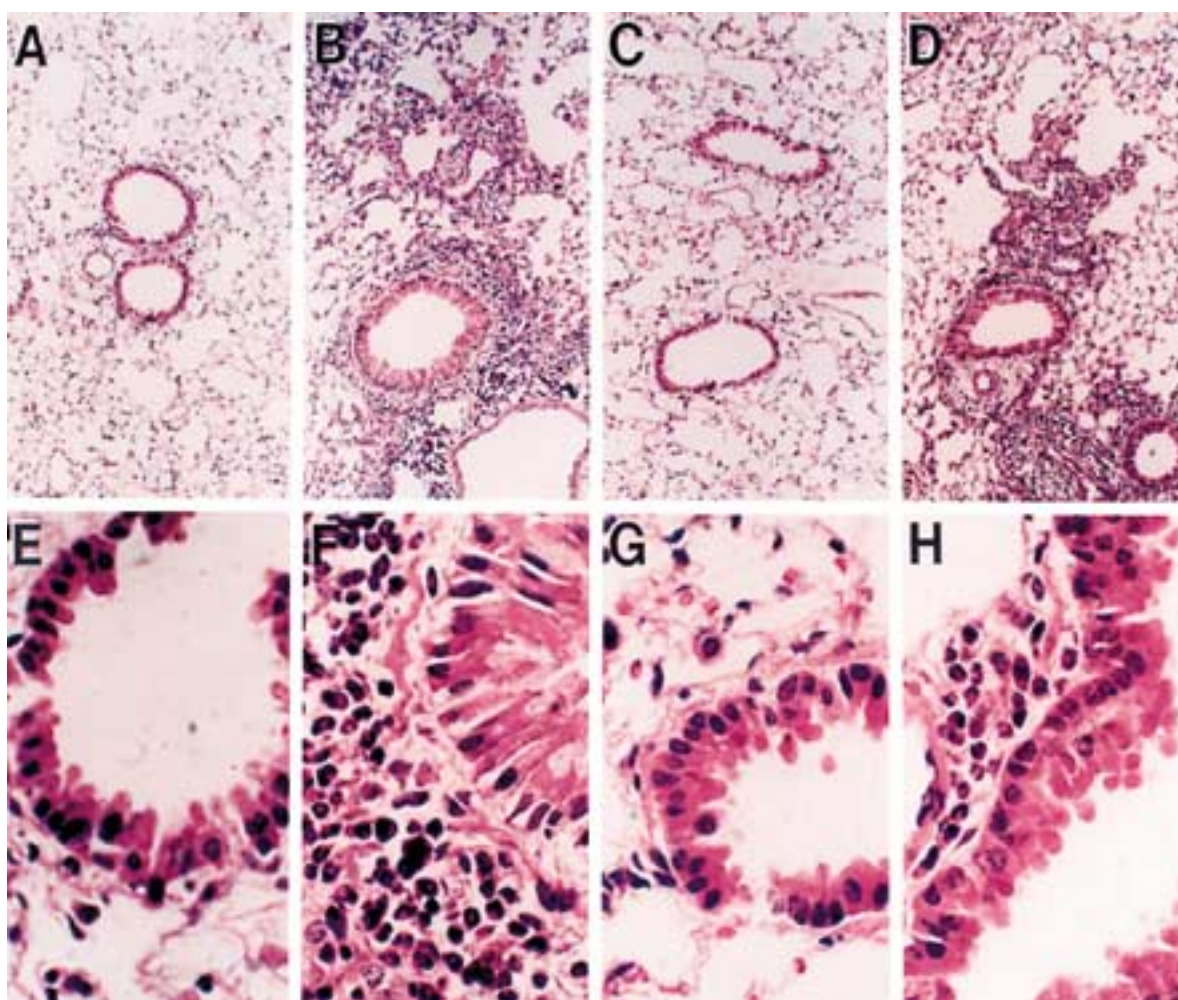


Fig. 1. HE-stained sections of murine lung. **A–D** $\times 160$ (original magnification); **E–H** $\times 1,000$ (original magnification). Saline control mice (**A, E**) demonstrate a bland parenchyma and bronchovascular bundle. In contrast, asthma control mice (**B, F**) display marked perivascular and peribronchial inflammatory infiltrate with eosinophils, lymphocytes, activated macrophages and other cells. This inflammation is nearly eliminated in the CpG-ODN-treated mice (**C, G**) but not in the control ODN-treated mice (**D, H**).

Preclinical Studies

Based on the epidemiologic data and our observations of the Th1 responses to CpG motifs, we hypothesized that administration of CpG ODNs may be an effective adjuvant in preventing manifestations of airway inflammation and altered physiologic responses in a murine model of asthma through suppression of Th2-mediated responses [17]. For these initial studies, we used a model in which C57BL/6 mice are sensitized to schistosome eggs and then challenged in the airways with soluble egg antigen (SEA) derived from the schistosome eggs. These mice developed marked airway eosinophilia, elevated BAL

fluid IL-4 (table 1). Histopathologic examination (fig. 1) demonstrated peribronchial and perivascular infiltration of eosinophils, lymphocytes, activated macrophages and other inflammatory cells. Inflamed mice had exaggerated physiologic responses to inhaled methacholine (fig. 2). When mice received injections of CpG ODNs at the time of sensitization, all of these manifestations of asthmatic responses were abrogated (table 1, fig. 1, 2). This was associated with an induction of the Th1 cytokines, IL-12 and IFN- γ , in BAL fluid.

Because of the association between protection from eosinophilic airway inflammation and the induction of

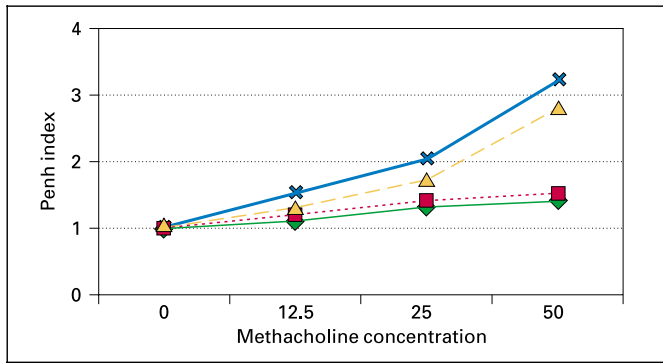


Fig. 2. Airway responses to methacholine inhalation challenge. Mice from various treatment groups are exposed to increasing concentrations of nebulized methacholine, after which they are assessed using a whole-body plethysmograph (Penh, Buxco). Asthma control mice (\times — \times) demonstrate marked bronchial hyperresponsiveness relative to saline control mice (\blacklozenge — \blacklozenge), this protection is nearly completely abrogated in the CpG-ODN-treated (\blacksquare — \cdots — \blacksquare), but not the control ODN-treated mice (\blacktriangle — \cdots — \blacktriangle).

Table 1. Schistosome egg asthma model: effects of treatment with CpG ODNs

Treatment group	BAL eos. ($\times 10^6$)	BAL IL-4 pg/ml	BAL IFN- γ ng/ml	BAL IL-12 ng/ml	Serum IgE μ g/ml
Saline control	ND	5 \pm 1*	0.02 \pm 0.01	0.6 \pm 0.2	0.66 \pm 0.38*
Asthma + control ODNs	2.2 \pm 0.9	78 \pm 18	0.12 \pm .08	2.0 \pm 0.8	3.04 \pm 0.94
Asthma + CpG ODNs	0.4 \pm 0.2*	23 \pm 3*	0.42 \pm 0.13*	5.5 \pm 1.9*	1.04 \pm 0.32*
Asthma control	3.5 \pm 0.8	118 \pm 29	0.02 \pm 0.01	1.8 \pm 0.7	4.10 \pm 0.54

n = 6/group; ND = none detected; * p < 0.01 vs. asthma control mice.

Table 2. Effect of CpG ODNs on protection against airway eosinophilia and bronchial hyperreactivity in mice who were sensitized to schistosome eggs in the presence or absence of IFN- γ and/or IL-12, and in the presence or absence of CpG ODNs

Mouse type	Antibody T _x	CpG ODN T _x	BAL eos.	Penh ₅₀
C57BL/6	—	—	1.6 \pm 0.4 $\times 10^6$	3.6 \pm 0.2
C57BL/6	—	+	0.2 \pm 0.1 $\times 10^6$	1.2 \pm 0.3
C57BL/6	anti-IFN- γ	+	0.1 \pm 0.1 $\times 10^6$	1.1 \pm 0.2
C57BL/6	Anti-IL-12	+	0.2 \pm 0.3 $\times 10^6$	1.2 \pm 0.2
IFN- γ KO	—	+	0.2 \pm 0.3 $\times 10^6$	1.4 \pm 0.2
IL-12 KO	—	+	0.3 \pm 0.3 $\times 10^6$	1.3 \pm 0.3
DKO	—	+	0.3 \pm 0.2 $\times 10^6$	1.6 \pm 0.3

Penh₅₀ = Penh index (fold increase over baseline) after inhalation of 50 mg/ml of methacholine.

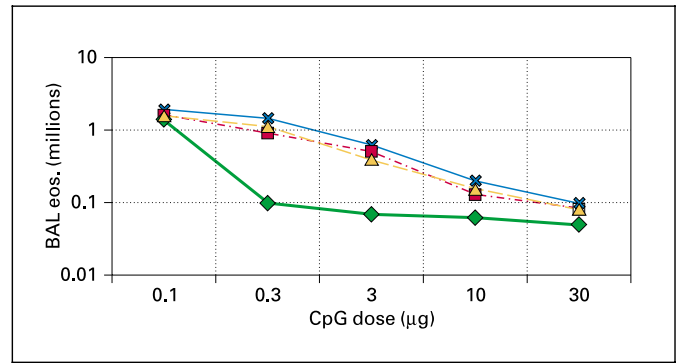


Fig. 3. CpG dose-response curve in inbred mice (\blacklozenge — \blacklozenge) and IFN- γ KO (\square — \cdots — \square), IL-12 KO (\triangle — \cdots — \triangle), and double (IFN- γ and IL-12) KO mice (\times — \times), all on C57 BL/6 background. Wild-type mice are substantially protected against the induction of airway eosinophilia when treated with amounts of CpG ODNs as low as 0.3 μ g; for equivalent protection, the KO mice require at least one log greater amounts of CpG ODNs.

Th1 cytokines in the airway, we next evaluated whether either IFN- γ or IL-12 was necessary for the protection offered by CpG ODNs [18]. For these studies, we used the same model of antigen-induced airway inflammation, but we carried out these studies in mice treated with anti-IFN- γ or anti-IL-12 antibodies as well as in mice whose gene for IFN- γ , IL-12, or both cytokines had been disrupted. To our surprise, we found that the administration of CpG ODNs was effective in preventing the development of both airway inflammation and of bronchial hyperresponsiveness in the absence of IFN- γ , IL-12, or both (table 2). When we carried out CpG dose-response studies, however, we found that there was approximately a log difference in sensitivity to the protective effects of CpG ODNs between wild-type inbred mice (C57BL/6) and mice deficient in either IFN- γ or IL-12 (also on a C57BL/6 background); the difference was even greater in double (IFN- γ and IL-12) knockout mice (fig. 3). These studies suggested to us that, although both of these Th1 cytokines played a role in the protection offered by CpG ODNs, neither alone nor in combination was essential for this protection. Undoubtedly there are redundant pathways through which these effects are mediated.

It was next important to determine whether CpG ODNs were effective in modulating attributes of asthma after the establishment of eosinophilic airway inflammation. For these studies, we adopted the schistosome-egg-induced murine model of asthma and administered ODNs, alone or in combination with antigen, at various time points. We first examined the effect of treatment fol-

Table 3. Effect of CpG treatment after sensitization on SEA-induced airway eosinophilia

Treatment	SEA (inhaled)	Harvest	BAL eos. ($\times 10^6$)
No treatment	weeks 2 and 3	week 3	2.6 \pm 0.8
CpG week 1 (+ eggs)	weeks 2 and 3	week 3	0.6 \pm 0.2
CpG week 2 (- eggs)	weeks 3 and 4	week 4	1.9 \pm 0.6
CpG week 5	weeks 3, 4, 6, 7	week 7	2.4 \pm 0.7
CpG + SEA week 5	weeks 3, 4, 6, 7	week 7	3.2 \pm 0.6
Saline (s.c.) weeks 4, 5, 6, 7	weeks 2, 3, 8, 9	week 9	2.9 \pm 1.2
SEA (s.c.) weeks 4, 5, 6, 7	weeks 2, 3, 8, 9	week 9	1.2 \pm 0.9
CpG + SEA (s.c.) weeks 4, 5, 6, 7	weeks 2, 3, 8, 9	week 9	0.2 \pm 0.3

n = 6/group. All mice sensitized to schistosome eggs (i.p.) on days 0 and 7 (weeks 0 and 1).

lowing sensitization but before airway challenge, and found that a single administration of CpG ODNs and SEA, but not CpG ODNs alone, was effective in blocking the subsequent airway inflammatory response to inhaled antigen in previously sensitized mice (table 3). We next evaluated the response to treatment in mice who had been

previously sensitized and challenged with antigen in the airways. Since a single treatment was ineffective in modulating eosinophilic airway inflammation (table 3), we instituted a course of immunotherapy treatment: 4-weekly subcutaneous injections of SEA in the presence or absence of ODNs. Following this therapy, mice were re-exposed to SEA. Mice treated with SEA injections demonstrated modest reductions in airway eosinophilia (table 3) compared with control mice (who received saline injections rather than active immunotherapy). Mice treated with SEA and CpG ODNs, however, had significant reductions in both airway eosinophilia (table 3) and bronchial hyperreactivity (not shown), suggesting that the CpG ODNs acted as an immunoadjuvant in downregulating established airway inflammation.

Conclusions

CpG ODNs have exhibited profound Th1-like immunomodulatory effects in both the prevention of antigen-induced asthma as well as treatment of previously established responses. These preclinical data have been reproduced by other groups, and there is strong support for the pursuit of clinical trials.

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